SEPARATION OF BIO-POLYESTER FROM BIO-POLYESTER-CONTAINING MICROBIAL CELL

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Inventor:	YOKOYAMA MASAKO
Applicant:	ASAHI CHEMICAL IND
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Abstract of **JP7031487**

PURPOSE:To provide a method for efficiently separating a bio-polyester in a granular state from microbial cells containing the bio-polyester. CONSTITUTION:The aqueous suspension of bio-polyester-containing microorganisms is mixed with an alkali in an amount of 1mmol-1mol/kg microbial cells and subsequently heated at 40-100 deg.C to separate the granular bio- polyester from the microorganisms.

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(71)出願人　000000033
旭化成工業株式会社
大阪府大阪市北区堂島浜１丁目２番６号

(72)発明者　横山　雅子
岡山県倉敷市潮通３丁目13番１　旭化成工業株式会社内

(74)代理人　弁理士　清水　猛　（外２名）

(54)【発明の名称】　バイオポリエステル含有菌体からのバイオポリエステルの分離方法

(57)【要約】

【目的】　バイオポリエステル含有菌体から、バイオポリエステルを効率よく顆粒状で分離する方法を提供する。

【構成】　バイオポリエステル含有微生物の水性懸濁液に１mmol／kg菌体～１mol　／kg菌体の量のアルカリを添加し、４０～１００℃に加熱して、微生物から顆粒状のバイオポリエステルを分離する。

9（1981）〕、3HBと4－ヒドロキシブチレート（4HB）との共重合体〔Y. Doi et al., Macromolecules, 21. 2722（1988）〕が挙げられる。細胞内に蓄積しているバイオポリエステルは、微小な顆粒として存在することが知られている。処理される細胞内のバイオポリエステル含有率（以下、ポリマー含有率という）は、高いほうが好ましい。一般に、乾燥菌体としてポリマー含有率が20重量%以上がよい。アルカリ添加量、処理時間、分離操作の効率、分離ポリマーの純度等を考慮すると、50重量%以上のポリマー含有率が特に好ましい。

【0007】水性懸濁液とは、培養終了後の培養懸濁液そのもの、または培養液から遠心等で分離した菌体を水に懸濁させたものを指す。使用するアルカリとしては、NaOHを始めとしてLiOH, KOH等を含めたアルカリ金属の水酸化物、あるいはNH, OHが用いられる。アルカリの使用量は1mmol／kg菌体～1 mol／kg菌体、好ましくは2．5mmol／kg菌体～200mmol／kg菌体、特に好ましくは50mmol／kg菌体～200mmol／kg菌体で、これを微生物の水性懸濁液に添加する。アルカリを添加後は、水性懸濁液を40～100℃、好ましくは60～100℃に加熱する。その温度での加熱時間は0．5～4hr、好ましくは1～2hrがよい。この時、撹拌や振とうにより、系内を均一化することは好ましい。以上の操作は、一般に1ないし2気圧の低圧下で行う。

【0008】このような操作を行うことにより、菌体を破壊し、バイオポリエステルを顆粒状で菌体から分離できる。菌体壁が破壊されると、核酸のような水溶性の高分子物質が細胞外に溶出するために、該懸濁液の粘度が上昇する。そのために、この一連の処理操作で使用する該懸濁液の菌体濃度は、その後の遠心操作、濾過操作等の分離操作の効率を考慮すると、乾燥菌体換算で100g菌体／l以下がよい。好ましくは30～100g菌体／lである。本発明により、アルカリ添加を行った微生物の水性懸濁液を加熱することにより、菌体壁を破壊し、バイオポリエステルを顆粒状で分離できる。

【0009】

【実施例】本実施例で用いた微生物は、アルカリゲネス＊属に属する微生物アルカリゲネス・リポリティカ（Alcaligenes lipolytica）AK201（特開平5－64592）で、培養後、P（3HB）を約50wt%含有している菌を遠心（8000rpm, 10min. 遠心分離機はKUBOTA製6810使用）によって培養液から分離後、ペースト状菌体に水を加えて40g菌体／lの水性懸濁液とした。この水性懸濁液を用いて、以下に示す実施例1，2および比較例1を行った。

【0010】実施例1，2および比較例1の操作で得たP（3HB）は、純度を調べるためにガスクロマトグラフィー、分子量分布の決定にゲルパーミエーションクロマトグラフィー（GPC）を用いて分析を行った。なお、ガスクロマトグラフィーには、実施例1，2および比較例1で得られた沈澱物を乾燥（105℃, 24hr）した後、メタノール／硫酸でメタノリシスして菌体内ポリエステルをモノマーのメチルエステルとしたものを分析して、ポリマー含有率を求めた。これは、〔H. Brandl et al. Int. J. Biol. Macromol., 11, 49－55（1989）〕に示される方法に従った。GPCは、試料（約100mg）中のポリエステルを熱クロロホルム150mlで抽出後、溶液を濃縮してヘキサンを加えて再沈し、沈澱を濾過、真空乾燥（2hr）して10mg／10mlのクロロホルム溶液にして測定した。

【0011】（実施例1）4．0mMとなるように0．1MのNaOH水溶液を加え、P（3HB）含有菌体の該懸濁液100mlを作成した。該懸濁液を密閉にした容器中で撹拌（100rpm）しながら80℃に加熱し、1hr撹拌を続けた。処理後の水性懸濁液を遠心分離（2700rpm, 10min）して沈澱物を得た。

（実施例2）8．0mMとなるように0．1MのNaOH水溶液を該懸濁液に添加するように変える以外は、実施例1と同様に操作した。

（比較例1）本例では、NaOH水溶液を添加しないこと以外は、実施例1と同様に操作した。

実施例1，2および比較例1の条件を表1に示す。

【0012】

【表1】

	アルカリ量	加熱温度
実施例1	4.0 mM	80℃（1hr）
実施例2	8.0 mM	80℃（1hr）
比較例1	無	80℃（1hr）

実施例，比較例について、ガスクロマトグラフィー、GPCより求めたポリマー含有率、分子量の結果を表2に示す。

【0013】

【表2】

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(71)Applicant : ASAHI CHEM IND CO LTD
(72)Inventor : YOKOYAMA MASAKO

(54) SEPARATION OF BIO-POLYESTER FROM BIO-POLYESTER-CONTAINING MICROBIAL CELL

(57)Abstract:
PURPOSE: To provide a method for efficiently separating a bio-polyester in a granular state from microbial cells containing the bio-polyester.
CONSTITUTION: The aqueous suspension of bio-polyester-containing microorganisms is mixed with an alkali in an amount of 1mmol-1mol/kg microbial cells and subsequently heated at 40-100° C to separate the granular bio- polyester from the microorganisms.

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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] The separation approach of the biotechnology polyester content biomass characterized by adding the alkali of the amount of 1 mmol/kg biomass - 1 mol/kg biomass to the aqueous suspension of a biotechnology polyester content microorganism, heating at 40-100 degrees C, and separating granularity biotechnology polyester from a microorganism to biotechnology polyester.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the separation approach of biotechnology polyester from the biomass of the biotechnology polyester which has biodegradability.
[0002]
[Description of the Prior Art] Although current and a plastic waste are processed by incineration, reclamation, etc., there are problems, such as warming of the earth and ground relaxation of a reclaimed ground, in these arts, respectively. Therefore, recycle system-ization is progressing with a rise of the social consciousness to plastics recycle. However, there is much what remains there being a limitation in a recyclable application, could not respond only by incineration, reclamation, and recycle as a plastic waste art as a practical question, and left in natural environment. Then, after abolition, it is incorporated by the cyclical change of materials of a nature, a biodegradable plastic from which a decomposition product does not serve as harmful matter attracts attention, and the development is furthered. As such plastics, especially the polyester that a microorganism generates within a biomass is expected that it is included in the carbon cycle process of a nature, and stabilization of an ecosystem is made. Moreover, also in the medical field, the implant material of recovery needlessness and the utilization as a drug carrier are possible.
[0003] However, in order to use this polyester as plastics, it is necessary to dissociate and to take out from the inside of the biomass of a microorganism. As an approach of obtaining biotechnology polyester from a biotechnology polyester content microorganism, a biomass is dissolved using the extraction method by organic solvents including chloroform, the following ******** soda (Williamson, D.H., and Wilkinson, J.F. (1958), J.Gen.Microbiol.19,198-203.), or a lysozyme, and the method of collecting the polymers which remained as granulation is learned. In addition, a biomass is destroyed by disconnection of the pressure of the high-pressure steam of the approach (JP,60-145097,A) and 100-degree-C ** which collect polymers by the dissolution of the biomass by specific enzymes other than a lysozyme etc., and there is the approach (JP,57-174094,A) of dividing into biomass fragment waste and a polymer etc.
[0004]
[Problem(s) to be Solved by the Invention] However, the solvent extraction method by chloroform etc. needs not only the extracting solvent concerned but the poor solvent for reprecipitation for a large quantity. Therefore, if it is going to reuse a solvent respectively, it is required to separate two sorts of solvents. Furthermore, since it is required to dry the whole biomass thoroughly in advance of solvent extraction generally and it also becomes requiring great heat energy, in order to produce biotechnology polyester industrially, much the facility for processes and energy are needed, and it is disadvantageous as a matter of fact. Although the fault of a solvent extraction method can be avoided when it processes with the following ******** soda, on the other hand, molecular weight lowering of polyester takes place (J. A.Remsay, E.Berger, B.A.Remsay and C.Chevarie(1990),J.Biotechnology Techniques 4, 4,221-226), and a problem arises in the quality of a polymer. Although it is effective for little experimental utilization, since an enzyme like a lysozyme is difficult to secure a large quantity, it

is not suitable for the mass production of biotechnology polyester. In the enzymatic process of JP,60-145097,A, the actuation before and behind enzyme processing becomes a multistage story, and, in addition, the room of an improvement is large for mass production. Since the approach by release of the pressure of JP,57-174094,A has not indicated the purity or yield of polyester which were obtained, its effectiveness is unknown. This invention aims at offering the approach of separating biotechnology polyester from the microorganism containing biotechnology polyester among an aquosity medium by heating at less than 100 degrees C by the low voltage force of 1 thru/or 2 atmospheric pressures without using an organic solvent.
[0005]
[Means for Solving the Problem] This invention relates to the aqueous suspension of a biotechnology polyester content microorganism preferably at the separation approach of the biotechnology polyester from a 1 mmol/kg biomass -1mol/kg biomass, and a 2.5 mmol/kg biomass - 200 mmol/kg biomass and the biotechnology polyester content biomass characterized by adding the alkali of the amount of a 50 mmol/kg biomass - 200 mmol/kg biomass especially preferably, heating in 40-100 degrees C, and separating granularity biotechnology polyester from a microorganism. The microorganisms used for this invention are bacteria (bacteria) which are accumulating biotechnology polyester in intracellular. For example, the bacillus of Alcaligenes (Alcaligenes), A.lipolytica Although strain, such as Pseudomonas (Pseudomonas), such as AK201 (JP,5-64592,A), A.eutrophus, and A.latus, a bacillus group (Bacillus), an azotobacter group (Azotobacter), and a Nocardia group (Nocardia), is shown, it is not limited to the class.
[0006] Here, biotechnology polyester points out the microorganism production polyester called polyhydroxy alkanoate [it is hereafter called P (HA) for short] including Poly D-3-hydroxy butyrate [it is hereafter called P (3HB) for short]. As typical examples other than P (3HB), the copolymer [P.A.Holmes et al (ICI), Eur.Pat.Appl.0052459 (1981)] of 3HB and D-3-hydroxyvalerate (3HV) and the copolymer [Y.Doi et al., Macromolecules, 21,2722 (1988)] of 3HB and 4-hydroxy butyrate (4HB) are mentioned. It is known that the biotechnology polyester accumulated in intracellular exists as minute granulation. The higher one of the intracellular biotechnology polyester content (henceforth polymer content) processed is desirable. Generally, 20 % of the weight or more has good polymer content as a dried cell. When an alkali addition, the processing time, the effectiveness of separation actuation, the purity of a separation polymer, etc. are taken into consideration, 50% of the weight or more of especially polymer content is desirable.
[0007] Aqueous suspension points out the thing which made water suspend the biomass separated from the culture suspension after culture termination itself, or culture medium by centrifugal etc. As alkali to be used, the hydroxide or NH4 OH of alkali metal including LiOH(s) including NaOH, KOH, etc. is used. the amount of the alkali used -- 1 mmol/kg biomass - 1 mol/kg biomass -- desirable -- a 2.5 mmol/kg biomass - 200 mmol/kg biomass -- especially, it is a 50 mmol/kg biomass - 200 mmol/kg biomass preferably, and this is added to the aqueous suspension of a microorganism. After adding alkali, 40-100 degrees C of aqueous suspension are preferably heated at 60-100 degrees C. The heating time in the temperature has preferably good 1-2hr 0.5 to 4 hr. At this time, it is desirable to equalize the inside of a system by stirring or the shaking. Generally the above actuation is performed under the low voltage of 1 thru/or 2 atmospheric pressures.
[0008] By performing such actuation, a biomass is destroyed and biotechnology polyester can be separated from a biomass by granularity. If a biomass wall is destroyed, since a water-soluble polymeric material like a nucleic acid will be eluted out of a cell, the viscosity of this suspension rises. Therefore, when the effectiveness of separation actuation, such as subsequent centrifugal actuation and filtration actuation, is taken into consideration, as for the cell mass concentration of this suspension used by this the processing actuation of a series of, or less 100g biomass / 1 are good at dried cell conversion. It is 30-100g biomass / 1 preferably. By this invention, by heating the aqueous suspension of the microorganism which performed alkali addition, a biomass wall is destroyed and biotechnology polyester can be separated by granularity.
[0009]
[Example] microorganism Alcaligenes RIPORITIKA (Alcaligenes lipolytica) AK201 (JP,5-64592,A) to which the microorganism used by this example belongs to Alcaligenes -- it is -- after culture

and P (3HB) -- about 50 wt(s)% -- by centrifugal (8000rpm and a 10min. centrifugal separator are 6810 made from KUBOTA activities), water was added after separation and to a paste-like biomass from culture medium, and the contained bacillus was used as the aqueous suspension of 40g biomass / 1. The examples 1 and 2 and the example 1 of a comparison which are shown below were performed using this aqueous suspension.
[0010] P (3HB) obtained by actuation of examples 1 and 2 and the example 1 of a comparison analyzed by using gel permeation chromatography (GPC) for the decision of a gas chromatography and molecular weight distribution in order to investigate purity. In addition, after drying the settlings obtained in examples 1 and 2 and the example 1 of a comparison (105 degrees C, 24hr), what carried out the methanolysis with the methanol/sulfuric acid, and made the polyester in a biomass the methyl ester of a monomer was analyzed to the gas chromatography, and it was asked for polymer content. This followed the approach shown in [H.Brandl et al.Int.J.Biol.Macromol., 11, 49-55 (1989)]. After an extract and a solution were condensed for the polyester in a sample (about 100mg) by heat chloroform 150ml, the hexane was added and reprecipitated, GPC was filtered, and the vacuum drying (2hr) of the precipitation was carried out, it was used as the chloroform solution (10mg / 10ml), and it measured it.
[0011] (Example 1) The NaOH water solution of 0.1M was added so that it might be set to 4.0mM, and 100ml of these suspension of P (3HB) content biomass was created. It heated at 80 degrees C, stirring this suspension in the container made sealing (100rpm), and 1hr stirring was continued. Centrifugal separation (2700rpm, 10min) of the aqueous suspension after processing was carried out, and settlings were obtained.
(Example 2) It was operated like the example 1 except changing so that it may be set to 8.0mM and the NaOH water solution of 0.1M may be added to this suspension.
(Example 1 of a comparison) In this example, it was operated like the example 1 except not adding a NaOH water solution.
The conditions of examples 1 and 2 and the example 1 of a comparison are shown in a table 1.
[0012]
[A table 1]

	アルカリ量	加熱温度
実施例1	4.0 mM	80℃ (1 hr)
実施例2	8.0 mM	80℃ (1 hr)
比較例1	無	80℃ (1 hr)

The result of a gas chromatography, the polymer content for which it asked from GPC, and molecular weight is shown in a table 2 about an example and the example of a comparison.
[0013]
[A table 2]

	ポリマー純度	Mn	Mw	Mw/Mn
実施例1	75.1 %	$1.96*10^5$	$3.53*10^5$	1.80
実施例2	8.05 %	$1.98*10^5$	$3.60*10^5$	1.82
比較例1	60.7 %	$1.79*10^5$	$3.36*10^5$	1.88

[0014]
[Effect of the Invention] By this invention, the new separation method which conquered the fault

1 thru/or 2 atmospheric pressures) of 100 degrees C or less in an aquosity medium without using an organic solvent.

[Translation done.]